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Host range restrictions of oncogenes: *myc* genes transform avian but not mammalian cells and *mht/raf* genes transform mammalian but not avian cells

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ABSTRACT **The host range of retroviral oncogenes is naturally limited by the host range of the retroviral vector. The question of whether the transforming host range of retroviral oncogenes is also restricted by the host species has not been directly addressed. Here we have tested in avian and murine host species the transforming host range of two retroviral *onc* genes, *myc* of avian carcinoma viruses MH2 and MC29 and *mht/raf* of avian carcinoma virus MH2 and murine sarcoma virus MSV 3611. Virus vector-mediated host restriction was bypassed by recombining viral oncogenes with retroviral vectors that can readily infect the host to be tested. It was found that, despite high expression, transforming function of retroviral *myc* genes is restricted to avian cells, and that of retroviral *mht/raf* genes is restricted to murine cells. Since retroviral oncogenes encode the same proteins as certain cellular genes, termed protooncogenes, our data must also be relevant to the oncogene hypothesis of cancer. According to this hypothesis, cancer is caused by mutation of protooncogenes. Because protooncogenes are conserved in evolution and are presumed to have conserved functions, the oncogene hypothesis assumes no host range restriction of transforming function. For example, mutated human proto-*myc* is postulated to cause Burkitt lymphoma, because avian retroviruses with *myc* genes cause cancer in birds. But there is no evidence that known mutated protooncogenes can transform human cells. The findings reported here indicate that host range restriction appears to be one of the reasons (in addition to insufficient transcriptional activation) why known, mutated protooncogenes lack transforming function in human cells.**

The transforming host range of oncogenic retroviruses is limited by the host range of the viral vector. In addition, the host range of some viral oncogenes appears to be limited intracellularly by the host species. For example, the *raf* gene of murine sarcoma virus 3611 (1) transforms murine embryo cells, but its avian sequence equivalent, the *mht* (2, 3) or *mil* (4) gene of avian carcinoma virus MH2, does not transform avian embryo cells (5). Experimental studies, testing the transforming host range of other viral oncogenes, have confirmed intracellular host restriction. For example, avian retroviral *myc* genes have not achieved morphological transformation of murine cells (6–9). Even after engineering retroviral *myc* genes into murine retroviral vectors, *myc* genes do not morphologically transform murine cells (10, 11). However, one study described a low percentage of morphological transformants among rat cells expressing a synthetic *myc* gene with a retroviral promoter. Transformation in this system also depended on cotransfection with a plasmid carrying a herpes virus promoter (50). Two other studies claimed evidence for transformation of some murine embryo cell types with synthetic retroviral *myc* genes, but they failed to distinguish between virus-induced proliferation of cells that are not normally growing in culture and morphological transformation (12, 13). In addition, the synthetic murine *myc* genes failed to induce tumors in mice—the most sensitive test for oncogenic transformation (13). Accordingly, all naturally occurring retroviral *myc* genes were found in oncogenic avian retroviruses (14).

Some retroviral oncogenes such as *src* and *fps* have a broad host range, transforming both avian and mammalian cells. It has been possible to isolate host range mutants of these retroviral transforming genes, and it has been argued that these mutations may affect the interaction between the transforming protein and cellular components involved in transformation (15, 16). In contrast, other retroviral oncogenes may have a more limited host range. This may reflect differences in expression of targets of transforming proteins.

The host range of retroviral oncogenes is also relevant to studies of human cancer in the light of the oncogene hypothesis. According to this hypothesis, mutation converts cellular genes, which share coding sequences (but not promoters) with retroviral oncogenes, to equivalents of viral oncogenes (14). Therefore these genes are called protooncogenes. (This study does not address cellular genes unrelated to retroviral oncogenes that have since also been called oncogenes; refs. 17 and 18). Because protooncogenes are conserved in all vertebrates and host range restrictions of corresponding viral oncogenes are practically unknown, the oncogene hypothesis assumes no host range restriction of transforming function for mutated protooncogenes. For example, mutated human proto-*myc* is assumed to cause Burkitt lymphoma, because avian retroviral *myc* genes cause cancer in birds. But there is no evidence that either retroviral *myc* genes or mutated proto-*myc* genes can transform human cells (9, 14).

Because mutated human protooncogenes lack transforming function for embryo cells (9, 17), a number of researchers have tried to prove the hypothesis indirectly by converting nontransforming protooncogenes to transforming retroviral counterparts and by testing their transforming function in susceptible nonhuman cell species. For example, instead of testing the hypothetical human proto-*myc* cancer gene in human cells, it was tested in avian cells after it had been converted to an avian retrovirus that looked very similar to natural oncogenic avian viruses with *myc* genes (19). Because the synthetic avian virus transformed avian cells, it was concluded that the mutated human proto-*myc* must be a human cancer gene. Likewise, proto-*abl*, a hypothetical human leukemia gene, was tested for transforming function in mice only after it had been engineered into a murine retrovirus that was nearly indistinguishable from a natural prototype known to cause leukemia in mice (20). Again it was concluded that mutated human proto-*abl* is a human cancer gene, because the synthetic murine virus had caused leukemia in mice. But there is no evidence that a retroviral *abl* gene can transform human cells in culture.

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Abbreviation: HaSV, Harvey sarcoma virus.

Indeed, such experiments bypass not only direct tests of the assumed transforming function of mutated protooncogenes, but also tests of possible host range restrictions of oncogenes.

Because the host range of retroviral oncogenes is relevant both to our understanding of the mechanism of transformation and to the validity of the oncogene hypothesis, we have reexamined the host range of an avian and an avian/murine retroviral *onc* gene, *myc* and *mht/raf*, in avian and murine cells. To bypass host restriction directed at the viral vector, the avian oncogenes were cloned into murine retrovirus vectors and murine oncogenes were cloned into avian vectors. It was found that transforming function of viral *myc* genes is indeed limited to avian cells and that of *mht/raf* is limited to murine cells.

MATERIALS AND METHODS

Cells and Viruses. Primary rat cells were prepared from 12- to 14-day-old embryos from pregnant Fisher rats. Mouse C3H10T1/2 (21) and primary rat embryo cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% newborn calf serum and incubated at 37°C in 5% CO_2. Cells were fed with fresh medium every other day.

Recombinant Murine Retroviruses Containing Avian MH2 and MC29 Virus-Derived *gag-mht*, *mht*, and *myc* Coding Regions. The recombinant H-gag-mht virus was constructed from a murine Harvey (Ha) sarcoma provirus cloned in pBR 322 and from an avian MH2 carcinoma provirus, termed L5MH2, that is also cloned in pBR 322 and has been described (5, 22). The HaS-provirus clone, termed pHa7, contains a partially redundant, circularly permutated provirus extending from an *Mst*II site near the 3′-end of the provirus through the 5′-long terminal repeat, the complete viral genome, and the 3′-long terminal repeat up to the *Pvu*I site near the 5′-end of the virus (14, 23, 24). This Ha-provirus was derived from a variant described previously (23) by joining the viral *Pvu*I site (blunted with T4 DNA polymerase) with the *Pvu*II site of pBR 322 and joining the blunted viral *Mst*II site with the blunted *Eco*RI site of pBR 322. The H-gag-mht recombinant virus was put together by ligating a 3.18-kb Δ*gag-mht* fragment, which was prepared by digesting L5MH2 with *Bst*EII, filling in the sticky ends with Klenow DNA polymerase, and then digesting with *Cel*II, to a 5.5-kb pHa7 fragment that was prepared by first digesting with *Sst*II and, after blunting the sticky ends with T4 DNA polymerase, digesting with *Mst*II (see Fig. 1). The sticky ends of *Cel*II and the *Mst*II site of pHa7 are complementary. The H-mht virus was generated from H-gag-mht virus by digestion with *Bam*HI and religation (see Fig. 1). H-myc virus was constructed by ligating the 1.79-kb *Hpa*I/*Mst*II fragment from L5MH2 to the 5.5-kb *Sst*II (blunted with T4 DNA polymerase)/*Mst*II fragment of pHa7 (see Fig. 1). The AKR-myc virus was constructed by ligation of the 1.79-kb *Bcl*I/*Bam*HI *myc* sequence from pMC29 (14, 22) and the 6.5-kb *Bgl*II-resistant fragment of pNB-AKR (14, 25) (see Fig. 1). The pNB-AKR clone was created by ligating an intact AKR provirus flanked by a *Nhe*I-bordered 5′-cell sequence (25, 26) and a 3′ *Bgl*II-bordered cell sequence into the *Nhe*I and *Bam*HI sites of pBR 322. The AKR-myc virus clone studied here was selected for the correct orientation of *myc* by digestion with *Pvu*I and *Not*I (see Fig. 1).

Transfection of Cloned Viral DNAs. Near-confluent C3H10T1/2 cells were split 1:4 the day before DNA transfection. The next day cells were transfected with 5–10 μg of recombinant provirus DNA and 1 μg of helper Moloney virus DNA, pZap (27), using the polybrene-dimethyl sulfoxide method (28). Cells were washed with phosphate-buffered saline (PBS), fed with fresh medium the next day, and split to 1:5 48 hr after transfection. Transformed foci were observed 14 days posttransfection. Cells transfected by nontransforming recombinant viruses were cotransfected with 0.5 μg of the neomycin resistance gene pLTR-*neo* (29). Neomycin-resistant cells were selected in culture medium supplemented with 1 mg of geneticin per ml (Boehringer Mannheim) as of 48 hr after transfection. Cells were fed with fresh medium containing geneticin every 4 days. Two weeks later, surviving colonies were collected separately and propagated. Only ≈25% of those colonies contained recombinant virus sequences in their genomes (as confirmed by Southern blot). Those were saved for further studies.

Virus Infection. C3H10T1/2 and primary rat cells were split 1:10 from confluent dishes the day before infection. Cells were infected with 0.5 ml of culture medium (0.45 μm-filtered) either from pH-gag-mht/pZap- and pH-mht/pZap-transformed cells or from cloned, geneticin-resistant pH-myc- and pAKR-myc-transfected and *myc*-positive cells. Polybrene was immediately added to a final concentration of 8 μg/ml. Twenty-four hours later, cells were washed with PBS and fed with fresh medium. Cells were scored for foci of transformed cells 8 days postinfection. Virus was purified from supernatant collected from near-confluent cultures every 3 hr, and viral RNA was extracted as described (30).

DNA, RNA, and Protein Analyses. Cellular DNA was prepared as described, and RNA was isolated from cells by the guanidinium isothiocyanate method (28). Viral RNA was isolated as described (24). DNA and RNA were electrophoresed and transferred to Hybond N membranes (Amersham) using standard methods (24, 28). *myc* and *mht*-sequences were detected by hybridization digoxigenin-11-dUTP-labeled DNA probes—i.e., a 0.54-kb *Cel*II/*Not*I fragment from the *myc* sequence and a 0.44-kb *Stu*I-resistant fragment from the *mht* region of MH2 (see Fig. 1), as described by the manufacturer (Genius system, Boehringer Mannheim). Cellular proteins were prepared from cells washed with PBS containing 1 mM phenylmethanesulfonyl fluoride (Boehringer Mannheim), solubilized in protein sample buffer (2% SDS/100 mM dithiothreitol/60 mM Tris, pH 6.8) containing 0.5 mM phenylmethanesulfonyl fluoride, boiled for 5 min and briefly sonicated to shear released DNA. Proteins were resolved by 10% SDS/PAGE. The proteins were then eletrophoretically transferred to a nitrocellulose membrane using a buffer containing 24 mM Tris base, 192 mM glycine, and 20% methanol for 2 hr at 4°C, with a constant current of 400 mA. After transfer, the membrane was rinsed with TBST (50 mM Tris·HCl, pH 7.5/150 mM NaCl/0.05% Tween 20), blocked with 5% nonfat dry milk/0.02% NaN_3 in TBST for more than 2 hr with gentle agitating, and incubated with a 1:500 dilution of rabbit anti-v-*myc* anti-sera (Caltag, South San Francisco, CA) in 1% bovine serum albumin (BSA) (fraction V, Sigma) in TBST for 2 hr with shaking. The membrane was then washed three times with TBST for 30 min. Following a 1-hr incubation with a 1:2000 dilution of phosphatase-conjugated anti-rabbit IgG (Sigma) in TBST containing 1% BSA, three washes with TBST, and one wash with TBS (TBST without Tween 20), protein bands were detected with bromochloroindolyl phosphate/nitro blue tetrazolium substrate according to Harlow and Lane (31).

RESULTS

Host Range of *mht/raf* Oncogenes. Because all oncogenic retroviruses carry cell-derived coding sequences linked to retroviral promoters (9, 32), the finding of a cell-derived sequence, termed either *mht* (5) or *mil* (4), in MH2 virus suggested that the *mht* gene was oncogenic (10, 22, 33). This hypothesis derived further support from the finding of a retroviral oncogene, *raf*, in murine sarcoma virus 3611, which shares all its cell-derived coding sequence with the *mht* gene of MH2 virus (2, 3).

However, a subsequent deletion analysis of MH2 proved that the *mht* gene had no transforming function (5), although it is highly expressed in avian cells (2, 34), and that the virus derives oncogenicity exclusively from its *myc* oncogene. Thus

the *raf* gene of murine sarcoma virus 3611 is a transforming gene for murine cells, but its avian sequence counterpart, the *mht* gene of MH2 virus, fails to transform avian cells.

The differential behavior of the two related retroviral genes could reflect a host range restriction of *mht/raf* in avian cells. An alternative hypothesis proposes that the potential transforming function of the *mht* gene for avian cells is suppressed by a 5′ *gag* gene (retroviral core protein) leader sequence or by 173 MH2-specific 5′-nucleotides of *mht* that are not present in the *raf* sequence of murine sarcoma virus 3611 (2, 22).

To distinguish between the host range and suppressor hypotheses, transforming function of the complete coding sequence of the Δ*gag-mht* gene of MH2 and of the *mht* sequence without the *gag* leader was analyzed in murine C3H mouse cells. For this test, a 3.18-kb Δ*gag-mht* sequence of MH2, flanked by a 5′ *Bst*EII and a 3′ *Cel*II site, and a 1.37-kb *mht* sequence, flanked by a 5′ *Bam*HI and a 3′ *Cel*II site, were cloned into a murine Harvey sarcoma virus (HaSV)-derived retrovirus vector (21, 24). The resulting recombinant viruses, in which the *mht* sequences replaced the native *ras* sequence of HaSV, were termed H-gag-mht and H-mht virus (Fig. 1). The plasmid clones of these recombinant viruses were transfected with helper Moloney provirus into C3H10T1/2 mouse cells (*Materials and Methods*). As can be seen in Table 1, the H-gag-mht clone produced 1.2 foci and the H-mht clone produced 1.8 foci per μg of DNA in the presence of helper virus and about 10 times less in the absence of helper virus 10 days after transfection (Table 1). Fig. 2 *C* and *D* show a focus of H-gag-mht virus-transformed C3H cells surrounded by untransformed cells at two magnifications. Under the same conditions, MSV 3611 virus DNA produced 11 foci per μg of DNA and 67 foci in the presence of helper virus DNA.

The supernatant medium of the H-gag-mht- and H-mht DNA-transformed C3H cells was then harvested and plated on rat embryo cells to test (*i*) whether H-gag-mht and H-mht viruses were produced and (*ii*) whether the resulting viruses were able to transform embryo cells. It can be seen in Fig. 2 *G* and *H* that the H-gag-mht virus was able to transform embryo rat cells. As expected, the incubation period between infection and focus formation was shorter (6 days) than the period between transfection and transformation (10 days; refs. 21 and 24). The H-mht virus also transformed rat embryo cells, just like H-gag-mht virus (data not shown). The titers of the transforming viruses generated by mouse C3H cells transformed with viral DNAs are reported in Table 1. Because the synthetic murine H-mht-viruses transform murine cells, but the Δ*gag-mht* gene in its native MH2 virus fails to transform

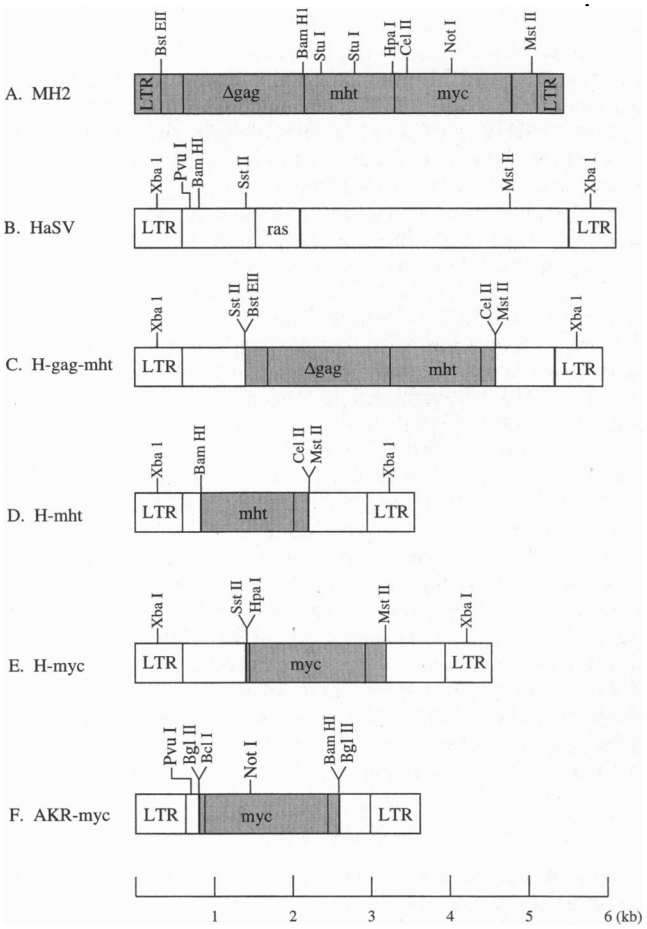

FIG. 1. Genetic structures of avian carcinoma virus MH2 (*A*), murine HaSV (*B*), and four recombinant viral DNAs: H-gag-myc (*C*), H-mht (*D*), H-myc (*E*), and AKR-myc (*F*). Three of the recombinant viruses consist of HaSV-derived vectors carrying either the complete coding region of the *gag-mht* gene (H-gag-mht), the *mht* coding region (H-mht), or the complete *myc* coding region of MH2 virus (H-myc). The AKR-myc virus contains the *myc* coding region of avian carcinoma virus MC29 in a vector derived from murine AKR virus. Restriction enzyme sites critical for the construction (*Materials and Methods*) and analysis of recombinant viruses are indicated. LTR, long terminal repeat; *ras*, coding region of the oncogene of HaSV.

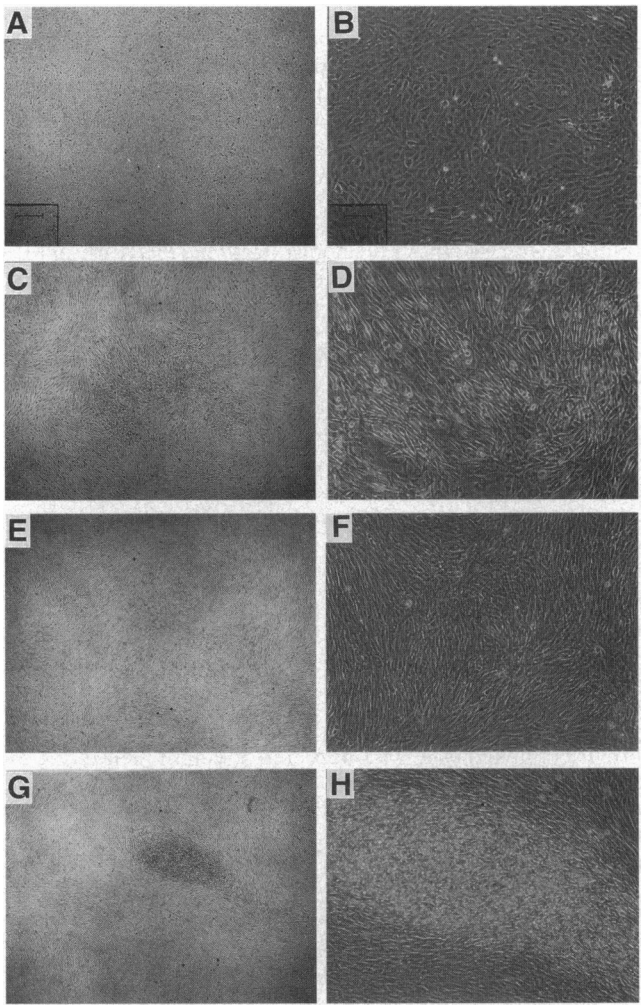

FIG. 2. The morphology of C3H10T1/2 mouse cells (*A* and *B*) and primary rat cells (*E* and *F*) infected with H-myc (Moloney helper virus) and of the same mouse cells (*C* and *D*) and rat cells (*G* and *H*) infected with H-gag-mht (Moloney helper virus). Bars = 400 μm (*A*, *C*, *E*, and *F*) and 100 μm (*B*, *D*, *G*, and *H*), corresponding to ×25 and ×100 magnifications, respectively.

Table 1. Transformation and virus production by cloned viral DNAs

Virus DNA used to transfect C3H cells	Transfection foci per μg of DNA	Infection, ffu*/ml
3611	11.0	
3611 (with helper virus)	67.0	1000
H-gag-mht	0.2	
H-gag-mht (with helper virus)	1.2	20
H-mht	0.2	
H-mht (with helper virus)	1.8	1000
H-myc	None	
H-myc (with helper virus)	None	None

*ffu, focus forming unit in C3H mouse cells.

avian cells (5), it is concluded that *mht*-transformation of avian cells is blocked by a host range restriction.

Host Range of *myc* Oncogenes. Because no natural, murine retroviruses with *myc* genes exist, we have re-investigated the potential of avian retrovirus-derived *myc* genes to transform murine cells after recombining these genes with murine retroviral vectors. Being within self-replicating murine retroviral vectors would maximize the dosage of *myc* genes in infected cells ≈100- to 1000-fold, compared with the dosage achieved by transfection with nonreplicating genes (21).

The murine *myc*-virus construct we designed closely resembles the highly oncogenic murine HaSV (Fig. 1). In this construct the native *ras* coding region of HaSV was replaced by a MH2-derived 1.79-kb DNA fragment, which includes the complete coding region of the *myc* gene from a *Hpa*I site 5′ of the splice acceptor to a *Mst*II site 3′ of the stop codon of *myc* (Fig. 1). The resulting synthetic *myc* virus was termed H-myc (Fig. 1). The plasmid clone of H-myc provirus (pH-myc) was transfected into C3H cells together with a plasmid clone of Moloney helper virus (pZap) as described above. However, no foci of morphologically transformed cells were observed within 2–4 weeks and over two to three cell transfers after transfection.

To distinguish between experimental flaws and host restriction of *myc* transformation the following experiments were undertaken. C3H cells were transfected again with pH-myc and pZap but also with the selectable drug-resistance marker pLTR Neo (*Materials and Methods*). If the ratio of unselectable to selectable DNA is kept high, this method allows for the selection of cells that have taken up unselectable DNA together with selectable DNA. Neo-resistant cells were then analyzed for the presence and replication of H-myc-virus and the transcription and translation of the viral *myc* gene.

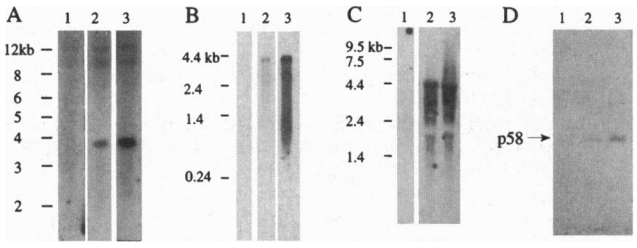

FIG. 3. Electrophoretic analyses of proviral DNA, virion RNA, intracellular RNA, and p58 *myc* protein of H-myc virus. *Xba*1-digested DNA (*A*, 10 μg of DNA per lane), virion RNA (*B*, 5 μg of RNA per lane), cellular RNA (*C*, 8 μg of RNA per lane), and protein (*D*, lysate from 2×10^5 cells per lane) of C3H10T1/2 mouse cells transfected with pZap DNA only (*A–D*, lanes labeled 1), of cells infected with H-myc/Moloney helper virus supernatant (*A–D*, lanes labeled 2), and of cells transfected by plasmid DNAs carrying H-myc and pZap DNA (Fig. 2) and long terminal repeat Neo (*A–D*, lanes labeled 3) were subjected to electrophoretic analyses and myc-specific sequences were detected as described. Selection of nontransformed, H-myc-transfected cells (Fig. 2) via Neo resistance is described. It can be seen in *A–D*, lanes 2 and 3, that H-myc DNA was integrated, producing virus, expressed intracellularly and translated, although the respective cells are phenotypically normal (Fig. 2).

It is shown in Fig. 3*A* (lane 3) that DNA of Neo-resistant C3H cells transfected with H-myc virus DNA contained the predicted 3.9-kb Xba-resistant H-myc-virus-specific DNA fragment (see Fig. 1).

Next we asked whether the synthetic H-myc virus was replicating. For this purpose C3H cells were infected with the supernatant of Neo-resistant cultures that carried H-myc DNA. Two weeks and two passages after incubation with that supernatant, the cells were analyzed for proviral H-myc DNA as described. It is shown in Fig. 3*A*, lane 2, that these cells contained the same 3.9-kb *Xba*I-resistant H-myc-DNA fragment as cells transfected by proviral DNA. This indicates that our virus construct was able to replicate in the presence of helper virus. Indeed the Southern blots shown in Fig. 3*A*, lanes 2 and 3, document that the concentration of proviral H-myc DNA significantly exceeded the concentration of endogenous proto-*myc* DNA that appears to electrophorese as 10-kb and 12.2-kb fragments after digestion with *Xba*I.

As expected from the DNA evidence for virus production, free virus purified from the supernatant of provirus-transfected and virus-infected cells (Fig. 3*B*, lanes 2 and 3), and total cellular RNA from virus-producing cultures (Fig. 3*C*, lanes 2 and 3) both contained 4.4 kb H-myc virion RNA. Because of the retroviral poly(*A*) terminus of over 200 As (35), the RNA is slightly larger than predicted from the 3.9-kb proviral DNA (see Fig. 1). No such RNA was found in cells transfected with helper Moloney retrovirus (Fig. 3*B*, lane 1). Again the concentration of H-myc viral RNA much exceeded that of endogenous proto-*myc* RNA that ought to be present in infected and uninfected cells, but was below the limit of detection by our assay.

Although H-myc proviral DNA and viral RNA were abundant in C3H cells infected, but not transformed, by H-myc virus, it could be argued that the failure of our virus construct to transform was due to a technical flaw preventing translation. Therefore we attempted to detect the p58 *myc* protein produced by MH2 (36) virus in H-myc provirus-transfected cells and in virus-infected cells. It can be seen in Fig. 3*D*, lanes 2 and 3, that both provirus-transfected and virus-infected cells contained p58 *myc* protein. By contrast, no cellular proto-*myc*-encoded p58 *myc* protein was detected by our methods in pZap-transfected cells (Fig. 3*D*, lane 1). It would appear that the retroviral *myc* protein was produced, yet unable to transform, murine fibroblasts.

To minimize the possibility that the synthetic H-myc virus failed to transform fibroblasts due to a structural flaw of our construct, another murine *myc*-virus was constructed from avian carcinoma virus MC29 (37, 38) and murine AKR virus (39) (Fig. 1). For this purpose a 1.79-kb DNA fragment of MC29, flanked by a 5′ *Bgl*II site and a 3′ *Bam*HI site, was inserted into a AKR virus-derived vector (Fig. 1). Although the *myc* gene of MC29 virus is naturally fused with a *gag* gene-derived leader sequence (37), the *myc* sequence contains the same ATG translation start codon as the *myc* sequence of MH2 virus (2, 22). As can be seen in Fig. 1, this AKR-myc virus includes the complete *myc* coding region of MC29 virus. The AKR-myc provirus was transfected into C3H cells in the presence of helper Moloney provirus as described above for the H-myc provirus. However, no transformation was observed, confirming the result that retroviral *myc* genes cannot morphologically transform murine cells.

DISCUSSION

Our results confirm that the *mht* gene of the avian MH2 virus fails to transform avian cells, but, to our knowledge, reveal for

the first time that the *mht* gene transforms murine cells in a murine virus vector. This host restriction of *mht* was not appreciated previously, because it is masked in native MH2 virus by the presence of another, autonomous *myc* oncogene with an avian host range (5) (Fig. 1). Further, we confirm and extend earlier observations that the transforming host range of the *myc* gene with a native or artificial retroviral promoter does not include murine cells but is restricted to avian cells.

Host Range Restriction of Other Retroviral Oncogenes. Clearly host range restriction is not unique to retroviral *myc* and *mht/raf* genes. For example, the murine retroviral *ras* gene with a native or artificial retroviral promoter fails to transform human cells (40–42). However, human cells infected or transfected with *ras* genes linked to retroviral promoters have a higher than normal risk of transformation, although the role of *ras* in this transformation is uncertain (40–42). Further, there is no evidence that the *abl* gene of Abelson murine leukemia virus can transform any nonmurine cells, including human cells; this is the probable reason why Daley *et al.* (20) have tested suspected transforming function of a mutated human proto-*abl* gene in murine cells after cloning it into a murine retrovirus vector.

However, the host range of some retroviral oncogenes clearly exceeds that set by the replicating retroviral vector. For example, the murine retroviral *ras* gene transforms avian cells in an avian retrovirus vector (43). But viral *ras* is unable to transform human cells. Likewise, the *src* gene of avian Rous sarcoma virus cloned in a Moloney murine retrovirus vector transforms murine cells and causes solid tumors in mice (44, 45). Rous sarcoma virus has even been reported to cause tumors in monkeys but only after latencies of 3–9 months (46). But viral *src* is not able to transform human cells (47). Thus the host range of some retroviral oncogenes exceeds that set by the replicating viral vector, but does not appear to include all vertebrates, above all not humans.

In view of this it has been proposed that the host range of oncogenes results from a requirement for activation of multiple pathways in transformation. In some cell species, certain pathways are active and thus activation of a single cooperating pathway will be sufficient for transformation. In other cell species multiple pathways have to be activated for transformation (G. Steven Martin, personal communication). To prove this hypothesis it would be necessary to isolate cooperating genes.

Relevance of Host Range to the Oncogene Hypothesis of Cancer. Protooncogenes are conserved in evolution and thus it is generally believed that their function is also conserved (14). In contrast, our findings and those of others indicate that the transforming function of retroviral oncogenes is host cell-dependent. Because retroviral oncogenes and the corresponding protooncogenes encode the same or nearly the same proteins, oncogenic function of mutated protooncogenes, if it exists, should also be host cell-dependent. Thus the transforming function of a human mutant protooncogene cannot be inferred from its ability to transform cells of other species—either the aneuploid mouse 3T3 cells transfected with a mutant protooncogene (48, 49), or other animal cells infected with a mutant protooncogene that has been converted to an animal retrovirus. In addition, the transforming function of retroviral oncogenes critically depends on viral promoters that are 100- to 1000-fold stronger than those of the corresponding protooncogenes (9, 24, 32). A conclusive demonstration of the carcinogenic potential of a mutant human protooncogene will require either direct transforming function of human cells by transfection or inhibition of the transformed phenotype of human cancer cells by the elimination or neutralization of its function. To this date neither of these conditions have been met.

An alternative hypothesis proposes that cancer is caused by aneuploidy alone, involving abnormal numbers of normal chromosomes with or without mutated protooncogenes (32).

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